Virulence

Fimbriae, Pili, Flagella and Bacterial Virulence

Ali Hussain Al-Marzoqi

Rasha Hadi

Ahmed Saleem

2010

Fimbriae, Pili, Flagella and Bacterial Virulence

Main characteristic

- the ability of bacteria to bind to cells from potential host organisms.
- Fimbriated and piliated bacteria agglutinated erythrocytes in a fashion resembling classical hemagglutination and adhered to host epithelial cells
- Moreover, for some strains bacteria-induced **hemagglutination** was inhibited by the addition of the monosaccharide mannose. This suggested that **mannose** is used as a receptor for adherence and that the free mannose functions as a hapten
- For other bacteria-erythrocyte reactions hemagglutination was not inhibited by mannose implying another receptor selectivity in the binding reaction
- fimbriae or pili function as specific adhesive that aid bacterial colonization of mucosal surfaces.

Functions:

1. Fimbriae are known to bind plasma proteins and to initiate proteolytic cascades
2. others are capable of activating calcium influx and signal transduction cascades in host target cells
3. fimbriae have been shown to act as invasion and motility factors
4. bacterial flagella that typically mediate bacterial motility have also function bacteria adherence and in the initiation of proinflammatory responses.

Classification & biosynthesis

All adhesive factors show typically fimbrial in morphology they showed receptor – specific binding abilities. Fimbriae and flagella share the need to a polymer architecturally outside the ordinary bacterial anabolic machinery.

They classified according to:

1. **given assembly pathway**
2. **receptor specificity or antigenic variation**

1.Fimbriae Produced through the "Chaperone/Usher " Pathway

Types & Microorganism example:

The classical common **type-1 fimbriae** that mediate mannose-sensitive hemagglutination, and the **P-blood-group-antigen-binding P-fimbriae**, or **Pap pili**, are produced through the so-called ‘chaperone/usher’ pathway.

- fimbriae that belong to this ‘chaperone-usher’ family come in several different variants, and are not only defined to ***Escherichia coli***.

Function:

1. fimbriae are known to bind plasma proteins and to initiate proteolytic cascades.
2. capable of activating calcium influx and signal transduction cascades in host target cells.
3. fimbriae have been shown to act as invasion and motility factors.

Manufacturing:

1. Gene clusters that provide the fimbrial subunits, **protein chaperones** and outer membrane anchors for the fimbrial shaft, as well as specific fimbrial regulatory genes code for these fimbriae
2. **nine ‘biosynthetic’** genes and **two** fimbrial **regulatory** genes are included in the *E. coli* **pap gene** cluster responsible for the expression of **P-fimbriae** .
3. The **fimbrial are translocated to the periplasm** through the **housekeeping system**.
4. the **chaperones translocate fimbrial subunits** to the usher.
5. then initiates translocation and polymerization of the fimbrial subunits across the outer membrane
6. the P-fimbriae actually were composite fibers, The fimbrial fibers include at least two distinct functions:

a. the constitution of a filament

b. recognition of the receptor.

in addition to the major fimbrial subunit PapA,P-fimbrial filaments were found to contain **minor subunits** , including the **PapE**, PapF, **PapK** and **PapG** proteins located at the distal end of the fiber .

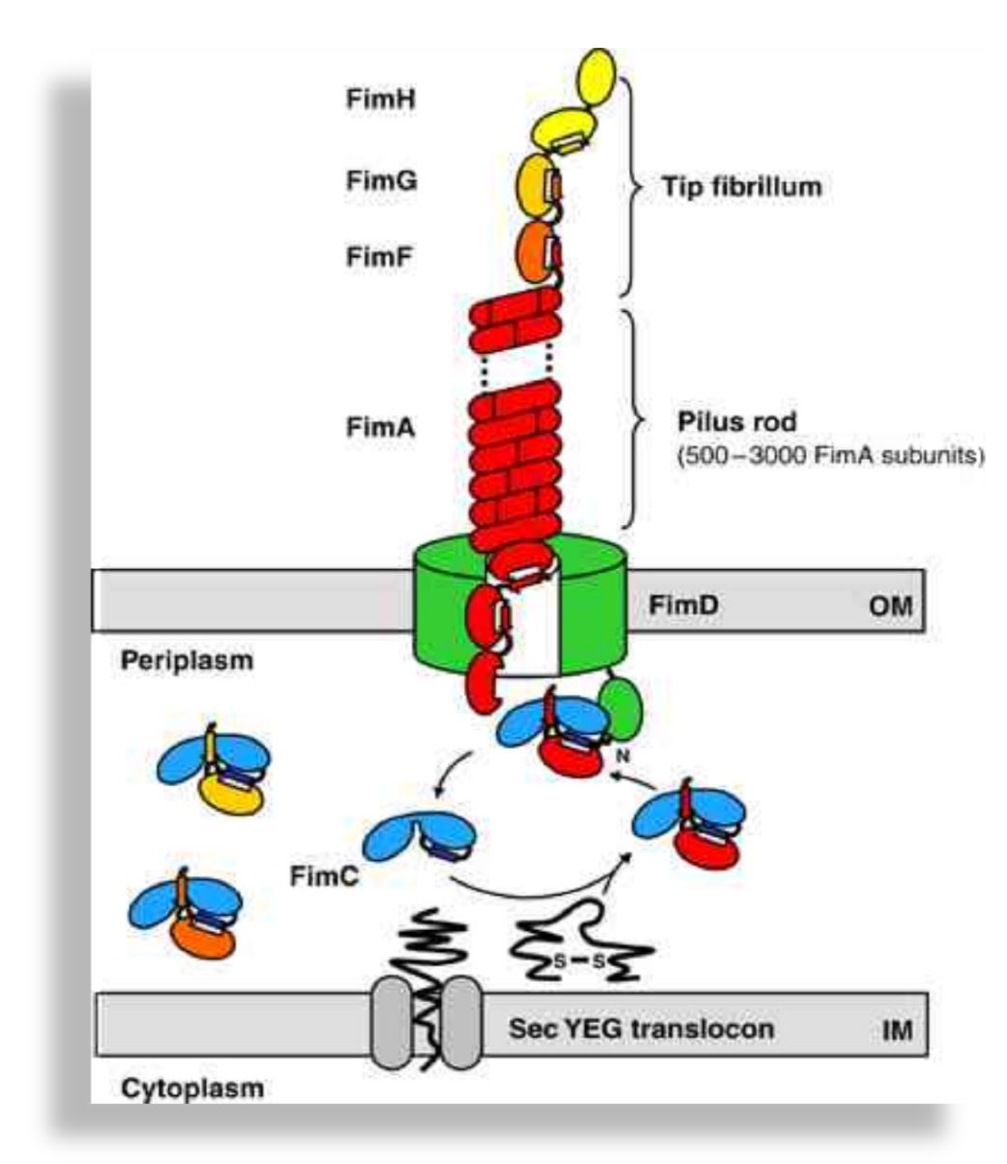
their function:

<u>The ability to bind the receptor resided in the PapG subunit, whereas other tip-located Pap proteins functioned as initiators of fimbrial polymerization and for adapting PapG to the fimbrial shaft.</u>

However, PapA alone forms the micrometer long shaft and hence substantially dominates preparations of isolated fimbriae. , type I fimbriae are also composite fibers.

Vip Outer-membrane **PapC** molecular ushers recognize periplasmic **chaperone-pilus subunit complexes**. The three proteins are, respectively:

1. recognize mannosides (FimH of the type I fimhriae)
2. Galα I — 4Galß (PapG of the P-fimhriae)
3. terminal N-acetyl-D-glucosamine (GafD or Fl7- G of the Fl7 fimbriae) share an immunoglobulin-like folding pattern .



Although all these fimbrial lectin proteins share the ability to bind a small carbohydrate epitope and to become integrated into the fimbrial filament

Fimbrial lectins are interesting candidate antigens for vaccine development. Due to the incomplete structural nature of the adhesin, vaccine trials have been conducted with adhesin-chaperone dimeric complexes. The FimH/FimC complex provided protection against uropathogenic E. coli in both a murine and a primate cystitis model

2.The CS1 Fimbrial Family

Types & Microorganism example:

The CSI fimibria forms the prototype of this class that includes several antigenic variants, including the classical **CFA/I fimbriae** of enterotoxigenic *E. coli* (ETEC), and **type II pili** of *Burkholderia cepacia.*

Fimbriae belonging to the class of the CS I fimbrial family are assembled in a manner that phenotypically resembles the 'chaperone-usher' pathway .

Manufacture:

1. The **CSI fimbrial** subunit **CooA** is translocated to the periplasm through a Sec-dependent path- way
2. then assisted by a protein **CooB** with chaperone-like function.
3. CooA is then fed to a larger transmembrane protein **CooC** concomitant with fimbrial polymerization.
4. polymerization needs the presence of minor fimbrial subunit protein **CooD**, which functions:- both as an *initiator* and the *lectin subunit*.

3.Fimbriae Produced Through The Extracellular Nucleator Pathway : Curli Organelles

- Many enterobacteria are capable of expressing elongated surface organelles, called AgfA fimbriae, with an "aggregative" and chemically robust character
- AgfA fibers appear **not as straight** but rather as twisted, curly structures and hence are referred to as " **Curli** " fimbriae.

Microorganism example:

- Curli fibers of ***E. coli*** and ***Salmonella enterica*** **sv Tyhpimurium** are cooled for by the cfg gene cluster. The cluster consists of two divergently transcribed units that include the csgABC and csgDEFG genes, respectively.
- Although curli fibers are coded for genetic elements comparable in size to the P-fimbrial pap operon, the curli fiber polymerization process is apparently different.

- Interestingly, curli fibers show all the typical characteristic of **amyloid fibers**, such as the binding to the **dye Congo red**.

Manufacture:

- Unlike amyloid formation in human neurodegenerative disorders such as Alzheimer's disease, curli amyloids require a specific assembly machinery. Thus, Vip **the CsgA and CsgB fimbrial subunits appear to be secreted out from the bacteria, where after an interaction between the subunits in the extracellular compartment then leads to polymerization**.
- The CsgA subunit occurs in excess in the isolated filament, whereas in vitro both the CsgB subunit and the isolated CsgA subunit are capable of self-polymerization. Thus, as in analogy with type 1, P- and CSI fimbriae the assembly of curli organelles also involves a nucleator component (CsgB), proteins with apparent chaperone functions (CsgE), or a nucleator center (CsgG).
- As with type IV pili, curli fibers have a rather diverse spectrum of receptor targets.
- Curli fibers are reported to mediate binding to mouse small intestinal epithelial cells, in addition to various plasma and extracellular matrix proteins.
- participation of curli in the formation of biofilms.

4.Type IV pili

- **Type IV** pili are multifunctional adhesive structures expressed by a number of diverse microorgamism including *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*.
- Related structures have also been identified in *Vibrio cholerae* (toxin-coregulated pili, Tcp) and enteropathogenic *E.coli* (bundle-forming pill, **Bfp**).
- Vip type IV pill are composed primarily of a single protein subunit, termed **pilin**, which are arranged in a helical conformation with 5 subunits per turn. They share unusual amino-terminal **N-methyl Phenylalanine** and immunogenic **Carboxy terminal Di-Sulfade bound**
- Vip type IV pili can be glycosylated and/or phosphorylated depending on the bacterial species. They assembly in cytoplasmic membrane or periplasm. The assembly require **NBP** "Nucleotide-Binding protein" .
- Vip Prepilin peptidase and outer membrane protein are essentially in **protrusion**.

Vip

type IV pili may share evolutionary origins with filamentous bacteriophages , and with genes required for bacterial type II protein export and DNA uptake systems; while others **; evolutes through their need to produce Sticky surface-located adhesive organelles**

Type IV pili of *Neisseria* are composed of a major pilus subunit and several other pilus-associated proteins, which have different functions in pilus assembly and adhesion One of these protein is **PilC**, which is associated with the tip and the shaft of the pili and the basal part in the outer membrane

Examples of The Role of Fimbriae in Pathogenesis of Mammalian Hosts

Chaperone/Usher Fimbriae and Urinary Tract Infection

Adhesion

- The ability to express certain types and sets of fimbriae seems overrepresented among urinary tract isolates of *E. coli*.
- The expression of type I fimbriae appears to be both an important:

1. colonization factor
2. factor contributing to the persistence in the bladder epithelium.

- *Vip* The pattern of mannose binding by the protein FimH is somewhat different among commensal and UTI *E. coli*:

  UTI isolates seem capable of binding D-mannose whereas commensals seem to prefer trimannoside structures

Vip P-fimnbriae recognize the core GalαI — 4Galß contained in blood group antigen-carrying globoseries glycolipids. the class II G adhesion recognizes most members of GalαI — 4Galß-containing globoseries glycolipids and has been considered important for kidney infection in persons with a non obstructcd urinary tract.

Beyond Adherence

- Besides mediating adherence to the urinary tract epithelium, type 1 and P-fimbriae have been implicated in the later phases of infection, and in **the generation of innate proinflammatory responses in the infected urinary tract epithelium**.
- **type I fimbriae** appear multifunctional in the pathogenesis of UTI; they:
    1. **mediate initial adherence**
    2. **invasion**
    3. **seem to participate in the formation of an intracellular biofilm.**
- Many types of fimbriae, including **type 1**, **type IC** and **P-fimbriae** have all been associated with the induction of proinflammatory responses in epithelial cells

Type I fimbriated *E. coli* induce cytokine expression from both A498 **kidney** epithelial cells as well as in **bladder** cell lines .

However, in bladder epithelial cells the majority of the **IL-6** response seems to derive from lipopolysaccharide (LPS) signaling through the CDI4-TLR4 pathway. LPS-recognizing Toll-like receptor TLR4 has been implicated in P-fimbria-induced host responses. Binding of P-fimbriated bacteria to A498 cells also caused an up regulation in the expression of TLR4mRNA suggesting that one function of P-fimbria-mediated host cell responses might be to modify the surface of the host cell to promote the infection.

- Mechanism of **P-fimbriae** are binding of bacteria causing release of ceramide in target cell concomitant with activation of cytokines release.

Type IV pili in Sequential Attachment and Invasion of Pathogenic *Neisseria*

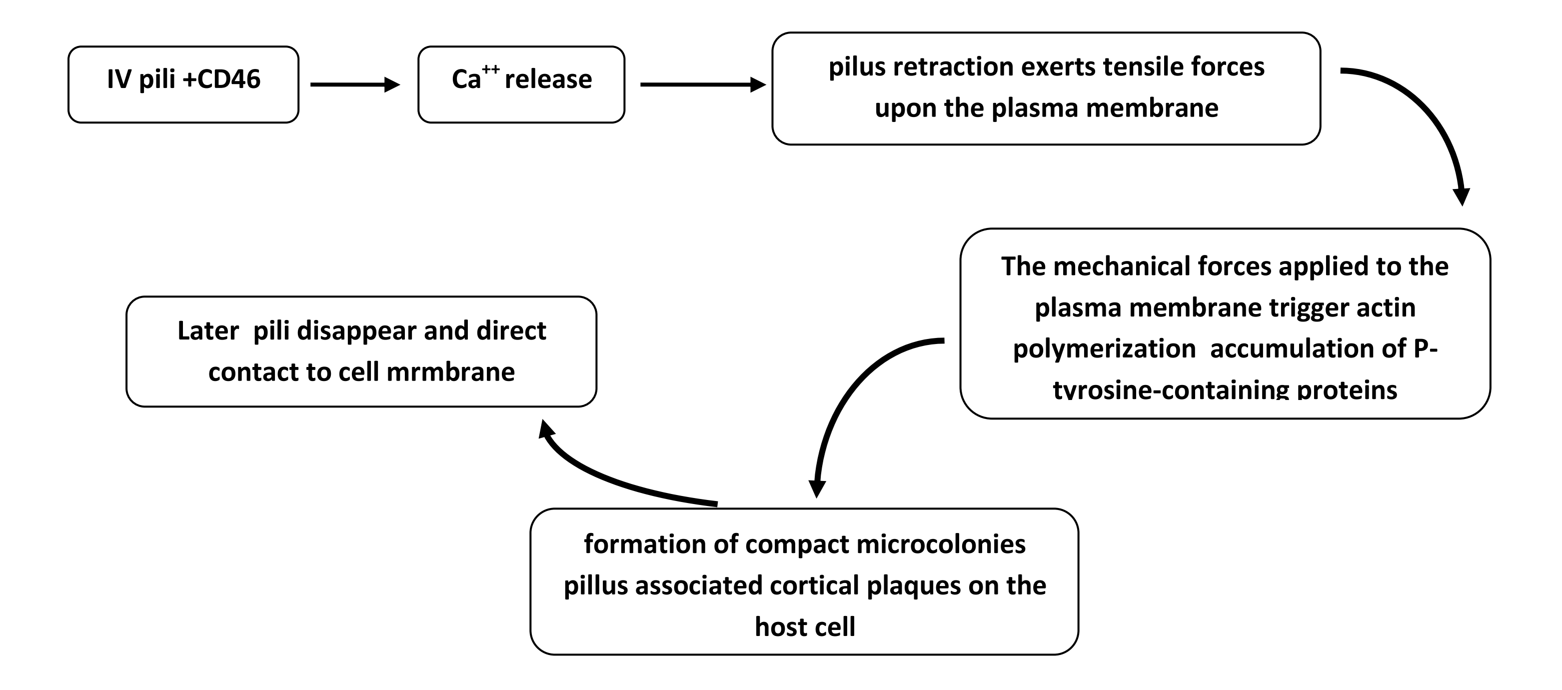
Adhesion

- The important initial interaction between pili of *Neisseria* and its host cell occurs through the receptor molecule **CD46**, a human cell surface protein involved in the regulation of complement activation.
- During initial contact between bacteria and cells, pilus retraction exerts tensile forces upon the plasma membrane.

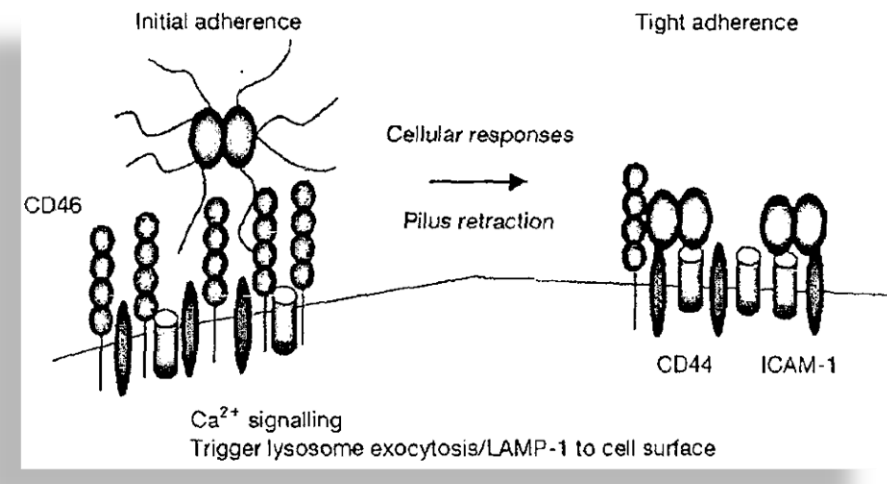
- The mechanical forces applied to the plasma membrane trigger actin polymerization accompanied by accumulation of phosphotyrosine-containing proteins, which leads to the formation of compact microcolonies.

Fig. Initial adherence of type IV piliated *Neisseria* involves initial contact with cell surface receptors followed by cell signaling leading to tight adherence and invasion of host cells. Failure in the pilus retraction events and/or host cell signalling leads to lost or changed adherence patterns, and a loss of ability to enter and invade host cells.

- type IV pill do not only simply anchor the bacteria at the cell surface, they initiate a **multistep adhesion cascade**,
    1. which starts with a loose adherence and ends with the attachment of bacteria.
    2. Type IV pili also assist in the formation of biofilms that may support further tissue colonization and protect the bacteria against antibodies and antibiotics.



Binding of Fimbrial Structures to Extracellular Components



- primary role of fimbriae might be to mediate

adhesion and subsequent events through binding to specific structures on host (**epithelial**) cells, it has recently become evident that fimbriae can also bind various **connective tissue proteins**, as well as **plasma** and **serum** proteins.

- The F17 fimbriae occur characteristically in *E.coli* isolates causing diarrhea and septicemia in newborn.
- F17 fimbriae mediate binding to the calf intestinal epithelium, which suggests a role for F 17 fimbria in the intestinal colonization. In addition, the Fl7 fimbria is capable of binding **plasminogen** and the extracellular matrix protein **laminin**.
- Binding to laminin is inhibited by the receptor N-acetyl-D-glucosamine, indicating that carbohydrate receptors on the extracellular matrix protein are recognized by the minor fimbrial lectin protein GafD.
- fimbriae may assist bacteria during tissue dissemination by directing them to extracellular matrix proteins, and by coating them with proteolytically active proteins that enable the bacteria to penetrate through the tissue.

Pili and Motility

- **Twitching motility** is flagella-independent movements of bacteria. Twitching motility occurs in a wide range of bacteria, and has been well studied in ***N. gonorrhoeae*** and ***P. aeruginosa***. it occurs on solid, wet surfaces and is mediated by **type IV pili.**
- Type IV pill serve as an initial bridge between bacteria and cells, and twitching motility allows bacteria to spread in the infected tissue.
- Twitching motility has been shown to be important for infection by ***P. aeruginosa*** as well as **for biofilm formation**, which appears to be involved in chronic infection.
- PilT, an ATPase associated with various cellular activities , seems to act as a molecular motor.

Phase Variation of Pilus Structures

- fimbriae and pili of the same type can be expressed as antigenic variants. For example, separate strains of UTI *E. coil* can express separate antigenic variants of the major fimbrial subunit protein , and a single strain can contain more than one P-fimbrial gene cluster.

- Furthermore, as different P-fimbrial gene clusters may contain separate papG alleles , and as P-fimbriae are subject to phase variation, the set-up provides *E. coli* with flexibility in terms of varying antigenicity and function of P-fimbriae.
- One extraordinary characteristic of the pathogenic *Neisseria* species is their capability to vary their surface pili the changing in the antigenic structures of surface proteins is certainly an important immune escape mechanism.
- the variation also modifies the function of these adhesions .

Flagella as Virulence Factors

- Like fimbriae, flagella are protein polymers, each flagellum consisting of thousands of flagellin monomers .
- These filaments are connected to the cell surface through the **'hook'** structure, and the **basal** structure that forms the rotation device and that traverses the bacterial cell wall.
- role of flagella is to **ensure motility**, either as **swimming** movement in liquid medium or as **swarming** on solid surface these are also applied in bacterial virulence . For example, flagella-mediated motility acts as a virulence function for *V cholerae* . For *V cholerae* and *H. pylori*, the role of flagella as virulence factors is also supported through which show an up regulation of motility genes in infecting bacteria.

For *P. mirabilis*, the swarming state involves a transition to a hyper flagellated state and an up regulation, the expression of selected virulence functions.

Vibrio parahaemolyticus and *Aeromonas* spp., apply two separate sets of flagella: **polar** and **lateral** sets .The different flagellar sets expressed by *Aeromonas* primarily associate with a **shift in motility**, the lateral set being used for swarming.

for *P mirabilis*, the switch to a swarming phenotype reflects a more fundamental alteration in the expression of the bacterial virulence potential.

The flagellar assembly pathway is related to the contact-dependent, so-called **type III protein secretion pathway** that is applied by many pathogens, like *Yersiniae*, for the translocation of bacterial virulence protein into host cell.